Synthesis of a Difluoromethylenephosphonate Analogue of Glycerol-3-phosphate. A Substrate for NADH Linked Glycerol-3-phosphate Dehydrogenase

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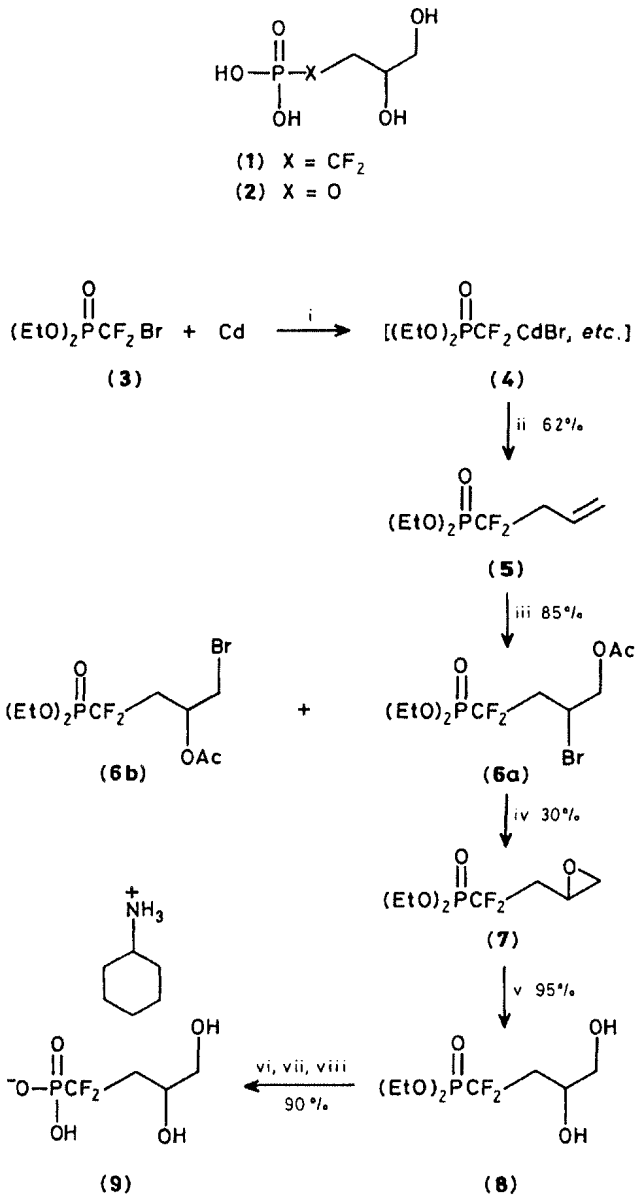
A synthesis of the cyclohexylammonium salt of (±)-1,1-difluoro-3,4-dihydroxybutylphosphonate (**9**) is described; (**9**) is a substrate for NADH linked glycerol-3-phosphate dehydrogenase.

The use of fluorine as a biological tool is increasing and has exciting possibilities; *e.g.* analogues of biologically significant[1,2] compounds can be prepared by replacing, H, OH, Me *etc.* with fluorine without introducing dramatic conformational changes. The rationale for such a strategy would become more convincing if the steric and electronic effects could be manipulated in such a manner that they complement each other to provide an analogue with isosteric and isoelectronic properties.

Phosphonates have been widely studied[3] as phosphate



Scheme 1. *Reagents and conditions*: i, THF, 65 °C, 3 h; ii, CH_2=$CHCH_2Br$, NaI (cat.), room temp., 20 h; iii, $Hg(OAc)_2$, Br_2, AcOH, 10 °C, 8 h; iv, KOH, MeOH, room temp., 5 h; v, HCl (cat.), H_2O/Me_2SO (2 : 1); vi, Me_3SiBr, Et_2O, room temp., 20 h; vii, H_2O; viii, $C_6H_{11}NH_2$.

analogues although their *in vivo* effects are compromised because their electronic properties deviate significantly from those of the phosphate group, but Blackburn and his co-workers have recently[4] shown that the composite steric and electronic effects of fluorophosphonates impart a much closer analogy to the parent phosphate. In order to explore the potential of this refinement we aim to synthesise a number of difluoromethylenephosphonates as phosphate analogues and test them in appropriate biological systems. We now describe the synthesis of the first of these compounds, 1,1-difluoro-3,4-dihydroxylbutylphosphonic acid (**1**) a structural analogue of glycerol-3-phosphate (**2**). Our approach involved (see Scheme 1) preparing the key allyl phosphonate (**5**) using a modification of the procedure[5] described by Burton and his co-workers. A combination of cadmium and bromodifluoromethylphosphonate (**3**) generates (**4**) as the major species of an organocadmium reagent. Reaction of (**4**) with allyl bromide gave the required derivative (**5**) and then treatment of (**5**) with bromine and mercuric acetate provided a 50 : 50 mixture of bromo-acetates (**6a** and **6b**). The oxirane (**7**) was obtained in moderate yield when this mixture was stirred in methanolic potassium hydroxide, acid-catalysed ring opening afforded diol (**8**)[6] which was treated with bromotrimethylsilane[7] for conversion into the corresponding di-trimethylsilyl ester. Aqueous hydrolysis then provided the free acid which was isolated after neutralisation as the cyclohexylammonium salt (**9**).

L-Glycerol-3-phosphate plays a central role in metabolism[8,9] and the biological properties of this novel difluoromethylene analogue are now under investigation. Preliminary studies using the glycolytic enzyme NADH linked glycerol-3-phosphate dehydrogenase show that (**9**) is turned over by the enzyme at one sixth of the rate† when compared to the natural substrate assuming conversion of only one enantiomer. Further enzymic studies are in progress and quantitative data will be described later.

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† Direct comparison of the initial rate between L-glycerol-3-phosphate and the phosphonate analogue (**1**) at 1.33 mM was determined under the following assay conditions: glycine (33 mM), hydrazine (33 mM), and NAD (4 mM); L-glycerol-3-phosphate dehydrogenase (16 units) was added at 25 °C, pH 9.6, to a final volume of 600 µl.